

WHAT IS CLAIMED IS:

1. A nucleic acid sequence comprising:

$P_x-S_x-B_n-(ZR)-\text{transport peptide}-(Z_1Z_2)-\text{protein}(Y)-(Z_1Z_2)-\text{protein}(Y_m)-T;$

wherein:

the nucleic acid codes for a fusion protein comprising a peptide encoded by transport peptide linked via a peptide encoded by a first  $Z_1Z_2$  to a protein encoded by protein(Y), which is linked to T when m equals zero, or when m does not equal zero, is linked to a peptide encoded by a second  $Z_1Z_2$  which is linked to a chain comprising at least one and up to 5 proteins encoded by protein( $Y_m$ ), which either correspond to the protein encoded by protein(Y) or can be different from the protein encoded by protein(Y);

the peptide encoded by transport peptide improves the rate of secretion of the protein encoded by protein(Y) and the protein encoded by protein( $Y_m$ ), when the protein encoded by protein( $Y_m$ ) is present;

$P_x$  comprises a promoter sequence;

$S_x$  comprises a nucleic acid sequence encoding a signal or leader sequence;

$B_n$  is 1 to 15 codons, when n is an integer from 1 to 15, or a chemical bond, when n = 0;

Z is a codon for lysine or arginine;

R is an arginine codon;

transport peptide comprises a nucleic acid sequence encoding a peptide that is transported across membranes;

Z<sub>1</sub> is a codon for lysine or arginine;

$Z_2$  is a codon for lysine or arginine;

protein(Y<sub>m</sub>) comprises a nucleic acid sequence encoding at least one and up

to 5 proteins that are produced and secreted by yeast when  $m$  is an integer from 1 to 5, or is a chemical bond when  $m = 0$ ;

protein(Y) comprises a nucleic acid sequence encoding a protein that is produced and secreted by yeast and whose biological activity, when protein(Y<sub>m</sub>) is not a chemical bond, is not impaired by a basic dipeptide extension encoded by the first or second Z<sub>1</sub>Z<sub>2</sub> or allows degradation of the basic dipeptide extension by carboxypeptidase; and

T is an untranslated expression-enhancing nucleic acid sequence.

2. The nucleic acid of claim 1, wherein the transport peptide encodes for hirudin or hirudin derivative.
3. The nucleic acid of claim 1, wherein protein(Y) encodes for one of mini-proinsulin, proinsulin, proinsulin derivative, interleukin, lymphokine, interferon, blood clotting factor, blood clotting factor derivative.
4. A fusion protein encoded by the nucleic acid of claim 1.
5. The fusion protein of claim 4, wherein the fusion protein comprises hirudin-derivative with two basic amino acid residues at its C-terminal end.
6. A multicopy vector comprising the nucleic acid of claim 1.
7. A plasmid comprising the nucleic acid of claim 1.
8. A host cell comprising the nucleic acid of claim 1 as a part of the host cell chromosome, as a part of a mini-chromosome, or extra-chromosomally.

9. The host cell of claim 8, wherein the host cell is a yeast.
10. The host cell of 9, wherein the yeast is selected from *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Hansenula polymorpha*, and *Pichia pastoris*.
11. A host cell comprising the multicopy vector of claim 6.
12. A host cell comprising the plasmid of claim 7.
13. A process of fermentative production of protein, comprising:
  - expressing the nucleic acid of the host cell of claim 8 to form the fusion protein in a supernatant of a cell culture; and
  - isolating the fusion protein from the supernatant of the cell culture.
14. The process of claim 13, wherein after expressing the nucleic acid, isolating the fusion protein comprises adjusting a pH of the cell culture to about 2.5 to 3.5 to precipitate non-desired protein.
15. The process of claim 13, further comprising separating the supernatant from the host cell, and after separating the supernatant from the host cell, the host cell is repeatedly cultured in fresh medium to form additional supernatant from each culture, and fusion protein is isolated from each additional supernatant.
16. The process of claim 13, wherein:
  - isolating the fusion protein comprises precipitating the fusion protein from the supernatant, and
  - the method further comprises removing the protein encoded by protein(Y) from the fusion protein, and concentrating the protein encoded by protein(Y) by one of microfiltration, hydrophobic interaction chromatography, and ion exchange chromatography.

17. A process of fermentative production of protein, comprising:  
expressing the nucleic acid of the host cell of claim 11 to form the fusion  
protein in a supernatant of a cell culture; and  
isolating the fusion protein from the supernatant of the cell culture.

18. A process of fermentative production of protein, comprising:  
expressing the nucleic acid of the host cell of claim 12 to form the fusion  
protein in a supernatant of a cell culture; and  
isolating the fusion protein from the supernatant of the cell culture.

19. A process for preparation of insulin, comprising:  
obtaining fusion protein by the process of claim 13, wherein the protein  
encoded by protein(Y) comprises proinsulin;  
releasing proinsulin into a reaction mixture by treating the fusion protein with  
trypsin and carboxypeptidase B; and  
isolating insulin from the reaction mixture.

20. The process of claim 19, wherein:  
transport peptide encodes for hirudin or hirudin derivative; and  
the hirudin or hirudin derivative is destroyed or biologically inactivated after  
the releasing of the prosinsulin or the isolating of the insulin.

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